

The immunoassay methods of this invention are based on the theory that normal individuals, that is, those without a mutation in a subject gene, will have 100% expression of that subject wild-type gene product and also 100% expression of a wild-type reference gene product. In contrast, an individual with a mutation in an allele of that same gene will in theory have only 50% expression of the subject wild-type gene product, while maintaining 100% expression of the reference wild-type gene product.

New Claim 61 is the same as original Claim 14, which had been incorporated in independent Claim 24. It is now separated from Claim 24 as a dependent claim again to point out with more particularity and clarity the subject matter regarded by the Applicant as his invention. Support for Claim 61 can be found in the application at page 7, line 6 to page 8, line 1; and at page 11, lines 22-25.

Applicant respectfully concludes that no new matter has been entered by the above amendments.

#### 35 USC 112, 1st Paragraph Rejection

Claims 24-28, 32-41, 43, 44 and 54-60 stand rejected under 35 USC 112, first paragraph "because the specification, while being enabling for methods where the subject genes are MLH1 and MSH2, does not reasonably provide enablement for practicing the claimed methods with any subject genes." [Office Action, pages 2-3.] Applicant respectfully traverses this

rejection, pointing out that the amendments to Claim 24 meet this rejection.

The Office Action mistakenly states on page 3 in the first full paragraph:

To practice the claimed invention one of skill in the art first needs to establish the biological association between the detected protein expressed by a subject gene or genes and specific diseases. The specification confines its teachings and examples to the association between MLH1 and MSH2 and the development of cancer. The specifications teachings are not adequate to support claims for detecting any disease or disease susceptibility trait. Thus, the (sic) to practice the claimed invention further and undue experimentation would be required.

Applicant respectfully points out that the claimed invention is not directed to any subject genes, but to only a subject gene or genes with an established biological association between the detected protein expressed by the subject gene or genes and a specific disease or specific diseases, and for which specific antibodies are available.

The Office Action then mistakenly also states at page 3 in the first full paragraph that "[t]he claimed inventions are drawn to methods for detecting any disease or disease susceptibility trait." Applicant respectfully points out that the Applicant's method is intended to detect the presence of an established disease, not to identify a new disease. The term

"detecting" is used in the sense of "identifying the presence of," and not in the sense of "discovering".

The methods of this invention could be used as a screening tool to detect new mutations that cause an about 50% decrease in the level of wild-type protein normally expressed by a subject gene, which mutations are associated with a specific disease or disease susceptibility trait. For example, one could test blood samples of a person known to have a specific disease with many antibodies, wherein each antibody is specific to a candidate protein. If one of such proteins is shown to be at an abnormally low level, said protein could be associated with a mutation that causes an about 50% decrease in the level of wild-type protein normally expressed by a subject gene. That association could then be investigated by other techniques.

However, such methods of detecting such new associations are not what is claimed in the subject claims. The question of detecting a previously unknown association between a mutation and a disease or disease susceptibility trait is by definition outside the scope of the instant claims.

The Office Action mistakenly states on page 4 in the top paragraph:

The specification provides an example with predicted ratio outcomes of a Western blot immunoassay, where the predicted ratio outcomes are based on the assumptions that a mutation in one gene will result in a decrease in expressed protein for that gene,

and that all organisms will only have one mutation in one of the genes. No ratio is predicted if an organism happens to have mutations in both of the subject genes.

[Emphasis added.] The Summary of the Invention at page 5, line 32 to page 6, line 2 reads in pertinent part:

. . . The immunoassay methods of this invention are also in this aspect premised on the assumption that germline mutations in two different genes of one individual are very rare.

[Emphasis added.]

Applicant recognizes that the present invention will not detect 100 percent of mutations that cause an about 50% decrease in the level of wild-type protein normally expressed by a subject gene, but that it will detect the predominance of them, and at a level comparable with other molecular tests. As pointed out in the Specification, for example, the inability to detect about 30% of HPNCC cases is about the same magnitude as reported for other molecular genetic tests (subject application, page 23, lines 24-25). In addition, "the immunoassay methods of this invention, by detecting the level of wild-type protein, may have greater sensitivity than the molecular genetic approaches, since they are able to detect, in addition to standard mutations, the mutations involving allelic loss, and mutations in the promoter, enhancer and splice site regions." [Subject application, page 4, lines 19-23.]

The Office Action on page 4 in the bottom paragraph mistakenly, in respect to the instant invention, states that "the art of genetic mutation and its relationship to disease or disease susceptibility does not appear to be predictable." Applicant respectfully submits that the types of genetic mutations that are the subject of detection in the instant claims can be correlated with certain diseases or disease susceptibilities. "[M]utation or loss of a single allele may be sufficient to exert a cellular phenotype that leads to tumorigenesis without inactivation of the second allele. This gene-dosage effect is called haploinsufficiency and has been demonstrated by at least two different experimental approaches. . . . Although it is well documented that gene-dosage effects cause developmental defects in model organisms and in certain inherited human diseases, their importance in tumor biology has been overlooked." [Fodde and Smits, Science 298:761-763 (2002); emphasis added.] One class of mutations causing haploinsufficiency are truncation-causing mutations, or "mutations that generate shortened proteins," which are associated with "the near-exclusive highlighting of disease-causing mutations." [Den Dunnen et al., Hum Mutat, 14(2):95-102 (1999); emphasis added.]

The claimed invention is not directed to any subject genes, but only to those genes for which germline mutations,

that are "selected from the group consisting mutations that cause an about 50% decrease in the level of wild-type protein normally expressed by a subject gene," are known to be associated with a disease or disease susceptibility trait. If the wild-type protein product of one of the subject genes of the claimed invention is present at an about 50% dosage, i.e. at about 50% of the level of said wild-type protein normally expressed by the subject gene, then the assay methods of this invention will register said about 50% dosage of that protein in an abnormally low ratio to the amount of protein product expressed by the other subject gene or genes. Such an abnormally low ratio indicates that the patient carries a germline mutation that causes an about 50% decrease in the level of wild-type protein normally expressed by the subject gene, and that is known to be associated with a disease or a disease susceptibility trait related to the protein product.

The Office Action further states on page 4 in the bottom paragraph that "not all mutations result in a decreased expression of the protein" and cites to the case of p53 referencing to Passlick et al. However, that statement and reference are inapposite to the subject invention by definition. The methods of the instant invention are only detecting germline mutations that result in an about 50% decrease in the level of wild-type protein normally expressed by a subject gene. The

amendment to Claim 24, from which all the claims now under examination depend, claims detecting a disease or disease susceptibility trait associated with a germline mutation "selected from the group consisting of mutations that cause an about 50% decrease in the level of wild-type protein normally expressed by a subject gene."

Applicant respectfully submits that the claimed invention will not detect all germline mutations, wherein the mutation is associated with a disease or a disease-susceptibility trait, but only that particular subset of mutations that result in an about 50% reduced protein product levels. The claimed invention would not detect those mutations that result in increased expression of a protein, such as identified by Passlick et al., or those mutations that result in slightly diminished expression of a protein.

Subsequent assays may or may not be used to identify the precise mutation and/or a disease condition. The inventive methods require the use of an antibody directed to a subject protein whose presence at an about 50% decreased level is already known to be associated with a disease or a disease susceptibility trait.

The Office Action states on page 5, lines 1-5, that it appears that detection of protein immunologically does not correlate with detecting functional protein. Thus, it appears that before one of skill in the art

may practice the full scope of the claimed invention, one would have to engage in further, undue experimentation, to establish that a correlation existed between a given disease or disease susceptibility and a decrease in protein expression levels.

The term "wild-type protein" in the claims is used to mean "apparent wild-type length protein." As one of skill in the art knows, an immunoassay has limitations. The wild-type nature of a protein can be determined in the context of the immunoassay art as being comparably immunologically reactive as the known wild-type protein is, by its being bound by antibodies that are specific for the subject wild-type protein.

The Applicant respectfully submits that the question of whether the protein detected immunologically by the method of the present invention is functional, or not, does not affect the enablement of the claimed invention. If the apparent wild-type length protein detected by immunoassay is nonfunctional and is diminished by approximately 50 percent from normal, then a disease condition is detected from the diminished amount of the apparent wild-type length protein. If the apparent wild-type length protein is functional and is diminished by about 50 percent, then a disease condition is detected from the diminished amount of apparent wild-type length protein. But if, the apparent wild-type protein is non-functional but produced by a subject gene at a normal level, the instant invention would not be detecting such a mutation, since the subject methods are

only detecting mutations that cause an about 50% decrease in the level of wild-type protein normally expressed by a subject gene, and by definition a mutation that does not result in an about 50% decrease in wild-type protein product is outside the scope of the instant claims.

Applicant respectfully requests that the Examiner reconsider the subject 112, first paragraph rejection in view of the amendment to Claim 24, and the above remarks, and withdraw this rejection.

35 USC 103(a) Rejection of Claims 24-28, 31-43 and 58-60

Claims 24-28, 31-43 and 58-60 stand rejected "under 35 USC 103(a) as being unpatentable over Vogelstein et al. (WO 97/08341; published 6 March 1997) in view of Sommer (U.S. Patent 5,569,608; issued Oct. 29, 1996)." [Office Action, page 5; section 5]. Applicant respectfully traverses that rejection by first relying in the arguments made in the earlier Amendment dated April 2, 2002 concerning the subject invention. The arguments made therein concerning the simplicity of Applicant's invention as being evidence of its nonobviousness, as well as the case law supporting that statement are incorporated herein by reference.

If the present invention had been obvious to one of ordinary skill in the art, why did Vogelstein et al. not include

it in his invention? After all Vogelstein et al. states on page 1, second paragraph, that "there is a need in the art for a technique which is relatively simple to perform and which will detect a broad spectrum of mutations in genes of clinical interest." [Emphasis added.] Vogelstein et al.'s method cannot be considered "relatively simple to perform," whereas the subject invention provides methods that are "relatively simple to perform."

Vogelstein et al. does not, among other reasons, render the present invention obvious as Vogelstein et al. analyzes the products of a different genetic entity, one human allele, produced in a different cellular entity, an artificially constructed somatic cell hybrid, and which requires extensive additional experimentation to eliminate the possibility of artifacts.

The Office Action, in identifying the similarities between Vogelstein et al. and the Applicant's method, states in the paragraph bridging pages 5-6:

Vogelstein teaches a method for the detection of diseases associated with germline mutations. The genes may be MLH1 and MSH2 (page 4, lines 1-2). The method comprises detection of protein expression by Western blot (page 10 - page 11). Vogelstein teaches that a decrease in protein expression is associated with a mutation causing the disease and specifically uses the example of measuring MSH2 levels. The biological sample is peripheral blood lymphocytes, derived from a

body fluid, blood. The method is diagnostic or prognostic of cancer. MSH2 is a mismatch repair gene. The organism is human, a mammal and a vertebrate.

Applicant respectfully points out that the method of the present invention is based on directly analyzing the products of both alleles of a gene simultaneously, and in a normal cell, whereas Vogelstein et al.'s method is based on analyzing the products of each allele of a gene separately, and in an artificial construct, a somatic cell hybrid.

The method of Vogelstein et al. requires the confirmation of the presence of the subject human allele and of its normal behavior in an artificial construct. Vogelstein et al. indicates at page 4, in the bottom paragraph, that the methods disclosed therein require confirmation that the fused hybrid cells do in fact "carry a human chromosome which carries the gene of interest." The Applicant's method does not require such a confirmation, because the human cell will already carry a full complement of human chromosomes, and therefore the gene of interest.

When the Office Action refers at page 5 in the bottom paragraph to Vogelstein et al.'s method comprising the "detection of protein expression by Western blot (page 10-page 11)," Applicant respectfully points to a significant omission in that characterization - that is, that the proteins detected in

Vogelstein et al.'s Example 3 are "hybrid proteins." [Vogelstein et al., page 10, bottom full paragraph.]

Furthermore, Vogelstein et al. states that the hybrid cells need additional testing to determine if the human genes are behaving in the hybrid cell as they would in a human cell, and additional experimentation is needed for that determination. In the paragraph bridging pages 5-6 of WO 97/08341, Vogelstein et al. states:

It is a possibility that expression of the gene of interest might be inhibited in the hybrid cell environment. In order for the loss of expression of a gene of interest in the hybrid cells to be meaningfully interpreted as indicating a mutation in the human, one must confirm that the gene of interest, when wild-type, is expressed in rodent-human hybrid cells. This confirmation need not be done for each patient, but can be done once when the assay is being established.

The confirmation requires analysis of "flanking markers" proximal and distal to the gene of interest (page 5, top paragraph). In contrast, the Applicant's method bypasses those additional lengthy and complicated procedures, as the genes in question are already behaving normally, in their normal environment of human cells. In addition, because of the unpredictability of the art of somatic cell fusion and resulting gene expression, Applicant's method eliminates possible sources of unpredictability.

Vogelstein et al.'s method is further complicated by the presence of rodent gene products. Vogelstein et al. state on page 5, second full paragraph,

If an immunological method is used to detect the protein product of the gene of interest in hybrids, it is desirable that antibodies be used that do not cross-react with rodent proteins. Alternatively, the rodent genes which are homologous to the gene of interest can be inactivated by mutation to simplify the analysis of protein products. Such mutations can be achieved by targeted mutagenesis methods, as is well known in the art.

The Applicant's method eliminates the need for antibodies that do not cross-react with proteins of a second species, as the required antibodies are only exposed to proteins of a single species. The Applicant's method also does not require the alternative approach of inactivation of rodent genes by targeted mutagenesis. Therefore, the Applicant's method bypasses lengthy further experimentation necessary to prevent tracer antibody interaction with rodent proteins.

There is no suggestion in Vogelstein et al. of detecting an about 50% decrease in the amount of a wild-type protein expressed by a subject gene as signifying a germline mutation in one allele of that gene. While Vogelstein et al. claims the subset of mutations in one allele that result in diminished amounts of wild-type length protein on the Western blot, he does not show enablement for determining "a diminished

amount," as Applicant does by standardizing protein amounts with another subject gene product. Vogelstein et al. only refers to the reduced wild-type protein as indicating mutation resulting in the loss of the entire wild-type gene.<sup>1</sup>

Finally, the method of Vogelstein et al. takes an estimated four to six weeks to complete (Vogelstein et al., page 6; bottom paragraph), whereas the method of the Applicant takes about 24 hours (subject application, page 2, lines 19-20). Thus, the Applicant's method is a more direct, more efficient and more cost-effective approach that bypasses experimental steps that may result in loss of sample (e.g., hybrid cell culture contamination) or in a critical delay in patient treatment. The quantitative immunoassays of the present invention, unlike those of Vogelstein et al.,

can be done in any pathology laboratory and can be developed to be cost-effective to screen large numbers of individuals in a short amount of time. The assays can be performed quickly, and the results are immediately obtainable. Once the change in the product of a particular gene is identified by the immunoassay methods of the

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1. The Office Action mistakenly states at page 6, top paragraph that "Vogelstein shows that a decrease in MSH2 protein levels is associated with FAP." However, FAP is associated with an about 50% decrease in APC protein and not with such an about 50% decrease in the amount of a mismatch repair gene product. Again, Vogelstein et al. only refers to reduced wild-type protein as indicating a mutation resulting in the loss of the entire wild-type gene, and not of one allele or of the expression product of one allele.

invention, molecular genetic tests can then be employed to determine the precise location of the mutation.

[Subject application, page 5, lines 10-15.]

Applicant respectfully submits that one of skill in the art would not be using the four to six weeks of additional experimentation of Vogelstein et al. to detect germline mutations, if it were obvious that such a substantial time delay could be avoided. The methods claimed in the instant application address and overcome the confounding variables associated with immunoassays of normal human cells by comparing the concentrations of subject wild-type cellular proteins within the same sample, and calculating a ratio of the wild-type proteins expressed by each of two or more subject genes, preferably, two genes that have a related function and that are each candidates for having a known germline mutation that causes an about 50% decrease in the level of wild-type protein, for example, a known germline truncation-causing mutation or a mutation that causes allelic loss, which mutation is known to be associated with a disease or disease susceptibility trait.

In summary, the Applicant's methods can be distinguished from the methods of Vogelstein et al. in regard to the following points:

- 1) Vogelstein et al. fuses a human sample to hamster cells.

The present invention does not.

- 2) Vogelstein et al. analyzes products of one allele of a subject gene, isolated from a second allele, in hybrid cells. The present invention analyzes the products of both alleles of a subject gene in normal cells.
- 3) There is no suggestion in Vogelstein et al., as in the present invention, of detecting an about 50% decrease in the amount of a wild-type protein expressed by a subject gene as signifying a germline mutation in one allele of that gene.
- 4) Vogelstein et al. does not suggest the use of a ratio of the wild-type protein amounts of two subject genes to address the confounding immunoassay variables that are addressed by the Applicant's method.
- 5) The Applicant's method can be especially useful as a clinical screening test such that it can be performed inexpensively, in any clinical laboratory without specialized equipment, and rapidly, to assist in decisions such as surgery, etc., if necessary. Vogelstein et al.'s method, in contrast, requires more sophisticated laboratory equipment, technical expertise, and a substantially greater amount of time.

The Sommer reference describes a method of quantifying the concentrations of an analyte separated by

immuno-chromatography and labeled by a tracer that binds it during the separation, by comparing its tracer signal with a standard curve prepared from known concentrations of the same analyte with the tracer. The Sommer method of determining quantities of an analyte does not apply to the instant invention, as the latter does not determine absolute quantities of a subject gene product, but only determines relative quantities, by determining quantities relative to those of a second subject gene product and expressed as a ratio, to determine whether wild-type levels are present. While Sommer teaches that the calculation of a ratio in the quantification of immunological measurements is known in the art, the ratio referred to in the Sommer method is a ratio of bound to unbound tracer, which is not applicable to the instant invention.

Applicant respectfully submits that nothing in Sommer adds to the disclosure of Vogelstein et al. to render the present invention obvious.

#### 35 USC 103(a) Rejection of Claims 24 and 44

Claims 24 and 44 stand "rejected under 35 USC 103(a) as being unpatentable over Vogelstein et al. (WO 97/08341; published 6 March 1997) in view of Sommer . . . ; and further in view of Kinzler et al (U.S. Patent 6,048,701; issued April 11, 2000; effective filing date June 7, 1995)." [Office Action,

page 6, section 6] The Office Action at page 6 in the bottom paragraph states:

Claim 44 is drawn to methods where the protein detection is automated. Vogelstein and Sommer teach as described above, but fail to teach automated immunological methods. However, automated immunological methods are well known in the art as evidenced by the teachings of Kinzler.

Applicant respectfully traverses this rejection relying upon the above arguments in response to the obviousness rejection over Vogelstein et al. in view of Sommer. Kinzler et al. contains no disclosures that would bridge the lack of obviousness gap between the Vogelstein et al. in view of Sommer disclosure and the instantly claimed invention.

Kinzler et al. provides no suggestion on how one would massively alter the Vogelstein et al. methods to the simplicity of the instantly claimed methods. Kinzler contains no disclosure concerning calculating a ratio or ratios of the amounts of wild-type protein expressed by subject genes for use in detecting germline mutations.

Claim 44 is dependent on Claim 24. As the Federal Circuit stated in In re Fine, 5 USPQ2d 1596 at 1600 (Fed. Cir. 1988): "Dependent claims are nonobvious under Section 103 if the independent claims from which they depend are nonobvious." For the reasons stated in the section above, Claim 24, the independent claim upon which Claim 44 depends, is nonobvious

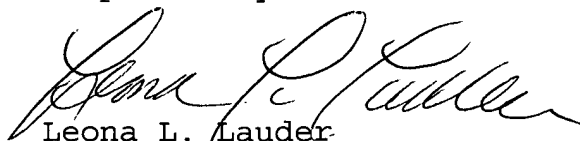
over Vogelstein et al. in view of Sommer. Since nothing in Kinzler et al. adds to the disclosure of Vogelstein et al., alone or in combination with Sommer, to render Claim 24 obvious, Claim 24 is similarly nonobvious in view of In re Fine (id.).

Applicant respectfully concludes that neither Vogelstein et al., Sommer nor Kinzler et al., alone or in any combination, render the instantly claimed invention obvious. Applicant respectfully requests that the Examiner review this rejection in view of the above remarks and case law, and withdraw this rejection.

#### CONCLUSION

Applicant respectfully concludes that the claims as amended are in condition for allowance, and earnestly requests that the claims be promptly allowed. If for any reason the Examiner feels that a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to telephone the undersigned Attorney for the Applicant at (415) 981-2034.

Respectfully submitted,

  
Leona L. Lauder  
Attorney for Applicant  
Registration No. 30,863

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## APPENDIX 2

### Claim 24 has been amended as follows:

24. (Thrice amended) A method of detecting a disease or a disease susceptibility trait in an organism, wherein said disease or said disease susceptibility trait is associated with a germline mutation in one of two or more subject genes, and wherein said germline mutation is selected from the group consisting of mutations that cause an about 50% decrease in the level of wild-type protein normally expressed by a subject gene ~~truncating-causing mutations and mutations that cause allelic loss comprising:~~

- (a) isolating a biological sample from said organism;
- (b) immunologically quantitating the amount of wild-type protein in said sample, that is expressed by each of the subject genes;
- (c) calculating the ratio of the amount of the wild-type protein expressed by one of said subject genes in said sample, to the amount of wild-type protein expressed by the other subject gene in said sample, or to each of the amounts of wild-type protein expressed by each of the other subject genes in said sample;
- (d) determining whether the ratio or ratios calculated in step (c) reflects or reflect an abnormally low level of a

wild-type protein expressed by either of the subject genes, or by any of the subject genes in said sample; and

(e) concluding that if the ratio or ratios calculated in step (c) indicates or indicate that there is an abnormally low level of a wild-type protein expressed by one of the subject genes in said sample, that that subject gene contains a germline mutation in one of its alleles, and that the subject organism is affected by the disease or the disease susceptibility trait associated with said germline mutation.